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Effect of Processing on Recovery of Polio Virus From Inoculated Foods

SUMMARY—The recovery of polio virus inoculated into a variety of foods was studied as a function of four food processing operations: freeze dehydration, "pasteurization" by exposure to Cobalt-60 gamma irradiation, refrigerated storage at 4°C, and storage at 20°C. In general, a decrease in virus recovery was noted following each of the operations. Complete loss in recoverability was observed only in highly acid foods (below pH 2.9) stored at 20°C for 96 or 168 hr. Use of a DEAE Sephadex chromatographic column was found to have potential application for separation of virus foods.

INTRODUCTION

INGESTION OF certain foods has been epidemiologically associated with outbreaks of several acute and chronic viral diseases (Becker, 1966; Berg, 1964; Cliver, 1966; Dack, 1964; Lemon, 1964). These findings suggest that low concentrations of viruses infective to man can gain entrance to foods during their production, processing, or preparation. While it is known that viruses do not multiply outside the living cell, little is known concerning the survival of virus in processed foods.

Information on the survival of virus in processed foods is necessary to an understanding of the epidemiology of food-borne virus diseases. Food processes designed for the destruction or control of bacteria, fungi, or molds cannot be assumed to remove or destroy food-borne viruses (Heidelbaugh et al., 1968a).

In the studies reported here, polio virus was inoculated into foods, and the recoverability of the virus was determined after the foods were subjected to a food processing operation. The processing operation employed was either: freeze dehydration, "pasteurization" by exposure to Cobalt-60 gamma irradiation, refrigerated storage at 4°C, or storage at 20°C. The foods were selected as representing types that would normally be considered for processing by the respective operation employed.

MATERIALS & METHODS

Viruses

Polio virus Type 1 strains, Mahoney (PIM), and LS$_c$2$_{ab}$ Sabin (PIS), were used in this study. The PIM stock suspension contained 1×10^8 plaque-forming units (PFU)/ml. The PIS stock contained 5×10^7 PFU/ml. The stock viruses were suspended in Eagle's basal medium (Eagle, 1955) with Hanks' balanced salt base (Hanks et al. 1949). Both stock suspensions were stored at −70°C until used. Virus dilutions were made in Hanks' balanced salt solutions (BSS).

Virus titration

The S_3 line of HeLa cells was used to propagate and titrate the virus by plaque count technique using a nutrient agar overlay. The cells were grown in suspension in Eagle's basal medium with Hanks' balanced salt base supplemented with 10% calf serum. The monolayers were prepared 24 hr prior to use by seeding plaque bottles with $4\text{–}6 \times 10^6$ cells in 10 ml of medium. The plaque bottles were tightly closed glass bottles presenting approximately 75×12 mm of flat surface area. Appropriate tenfold serial dilutions of the samples to be titrated were prepared using Hanks' BSS, and 0.2 ml of each dilution was added to each of 4 bottles. The monolayers were then incubated for 30 min at 37°C after which they were overlayed with Eagle's medium containing 1% agar. After 48 hr of incubation, the cultures were stained by adding 5 ml of 0.01% neutral red in Hanks' BSS to each bottle for 30 min. Plaques were counted 4–12 hr following staining.

Inoculation of food and preparation for recovery of virus. Each test sample of food consisted of 9 g of food inoculated with 1 ml of the virus stock being studied. Following inoculation and processing, each food sample was prepared for recovery of virus by taking the entire sample and blending in a sterile mortar and pestle while diluting with 90 ml of Hanks' BSS. This preparation was poured through sterile gauze following which the filtrates were centrifuged for 10 min at $2{,}700 \times G$ in 100 ml centrifuge bottles. The supernatant fluid was decanted and filtered through a Millipore filter (HA 47 mm 0.45μ) using a stainless steel wire support. All filtrates were titrated by determining the PFU/ml in triplicate or quadruplicate for each of 4 tenfold serial dilutions in Hanks' BSS. The resulting titers were reported as titer of food filtrate.

Preparation of Sephadex column eluate for virus titration. To study the usefulness of a Sephadex column for separation of virus from foods, some of the triturated and filtered inoculated food samples were titrated directly, and also after elution from a Sephadex column. The column was prepared using DEAE Sephadex A-25, medium grade, which was allowed to swell in water. Following swelling, the fines were removed and the gel was washed in sequence by 0.5*N* hydrochloric acid, water, 0.5*N* sodium hydroxide, water, and neutralized with 0.5*N* hydrochloric acid. The gel was then suspended in M/15 phosphate buffer (pH 7.5) and packed by pouring the suspension into 14 cm × 1.2 cm glass columns. No attempt was made to sterilize either the column or the gel.

Two ml of the food filtrate were entered into the top of the column and allowed to flow through the column by gravity. The elution of all columns was carried out at room temperature with M/15 phosphate buffer at pH 7.5. The first 3 ml eluate was discarded and the next 5 ml were collected (Giron, et al., 1964). Virus content of this eluate was determined and the resulting titer reported as titer of DEAE Sephadex eluate of food filtrate.

Recovery of virus from foods after freeze dehydration. Five foods were prepared according to guides for the production of foods intended for space flight feeding systems (Hollender et al., 1965). These foods were German potato salad (pH 4.9), beef and vegetables (pH 5.7), beef pot roast (pH 5.8), chicken with gravy (pH 6.1), and salmon salad (pH 6.1). Two 9 g samples of each of the five foods were inoculated with 1 ml of the stock suspension of PIM.

Following inoculation, each food was split into two samples, both of which were frozen by holding at −20°C for 24 hr. One sample of each frozen food was selected at random and freeze dehydrated for 24 hr at a pressure between 50 and 150 μ of Hg with a platen temperature of 21.8°C and a condenser temperature of −51°C. In this manner, all dehydration was performed on the samples simultaneously. Following dehydration the foods were immediately placed in individual jars with tightly fitting screw caps and held at −20°C until it was convenient for virus titration. It was assumed that no change in virus recoverability occurred during the −20°C storage. Randomly selected specimens of the dehydrated and nondehy-

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drated foods were titrated within a 4-week period following dehydration. The dehydrated samples were rehydrated immediately prior to titration by adding the quantity of water specified in the production guides. This entire sequence of procedures was performed three times and virus titers reported as arithmetic mean values.

Recovery of virus from fish fillets after exposure to Co[lt-60 gamma irradiation. Fourteen 9 g samples of frozen dressed Whiting fish were inoculated with 1 ml of the PIS stock suspension. Following inoculation the food samples were irradiated using a 6-kilocuric Cobalt-60 source (Hardy et al., 1965) of gamma irradiation. The samples were removed for virus recovery after 0, 150, 300, 375, 450, 525 and 600 kilorad total dose was obtained. Dose rate was 1 kilorad/min. Both filtrates and DEAE Sephadex column eluates of the filtrates of the irradiated fish were titered to determine virus recoverability.

Suspensions of the virus were prepared by dilution in Hanks' BSS to contain about the same titer as that recovered from the non-irradiated fish (4.2×10^6 PFU/ml). These suspensions were irradiated at the same time as the fish fillets with the following total dosages: 0, 75, 150, 225, 300, 375, 450, 525 and 600 kilorads. Following irradiation the suspensions were titrated both directly, and after passage through a Sephadex column.

Each fish fillet specimen and virus suspension was contained in an individual glass bottle packed in melting ice (0°C) at all times during the irradiation. This entire sequence of procedures was performed three times and virus titers reported as arithmetic means.

Recovery of virus from foods following storage at 4 and 20°C. Eight 9 g samples of each of five different foods were inoculated with 1 ml of stock suspension of PIS. The five foods consisted of four commercially prepared items and one experimental food. The commercial foods were canned jellied cranberry sauce (pH 2.7), reconstituted frozen concentrated orange juice (pH 2.9), mayonnaise (pH 4.2) and pork "junior food" (pH 5.6). The experimental food (pH .1) was a nutrient defined formula, which had been previously developed for physiological studies (Heidelbaugh et al., 1968b). The nutrient defined formula diet is formulated as follows; dry powdered skim milk, 40 g; sucrose, 20 g; lactose, 20 g; dextrose and maltose, 100 g; coffee whitener, 100 g; sodium caseinate, 17.5 g; sodium chloride, 1.5 g; and magnesium oxide, 0.1 g. This food was reconstituted before addition of virus. The pH of all foods was determined using a Beckman Zeromatic pH meter.

Following virus inoculation, half of the food samples were stored at 4°C and the other half at 20°C. Individual samples were stored for 48, 96, and 168 hr. Following storage the recoverability of virus was determined by titration of DEAE Sephadex column eluates of filtrates of the foods. This entire sequence of procedures was repeated once with virus titers reported as arithmetic means.

RESULTS

Polio virus survived the freeze dehydration process in all the foods tested.

Table 1—Recovery of polio virus[1] from foods after freeze dehydration[2]

Food	Titer without dehydration	Titer after dehydration
German potato salad	$3.0 \times 10^{6.3}$	1.5×10^3
Beef and vegetables	3.0×10^6	6.0×10^2
Beef pot roast	3.0×10^6	5.9×10^2
Chicken with gravy	2.6×10^6	2.9×10^2
Salmon salad	2.3×10^6	6.9×10^2
1 ml virus stock in 9 ml Hanks' balanced salt solution	4.0×10^6	2.0×10^3
1 ml virus stock in 9 ml water	1.5×10^6	4.0×10^1

[1] Nine g samples of food inoculated with 1 ml of virus stock suspension containing 1×10^6 plaque forming units (PFU)/ml polio virus type 1 Mahoney strain in Eagle's basal medium with Hanks' balanced salt base.

[2] Freeze dried at 50 to 150 μ of Hg pressure, condenser temperature −51°C, platen temperature 21.8°C.

[3] Mean PFU/ml of quadruplicate titrations of each sample from three studies.

The results of these studies are presented in Table 1. The average titer of recoverable virus was between 3 to 4 logs less in the freeze dehydrated food compared to the food that was not dehydrated. The loss in titer of the stock suspension diluted in Hanks' BSS was equivalent to the loss in the foods.

In the studies of recovery of polio virus from irradiated fish fillets, it was observed that a total dosage of 600,000 rads was sufficient to produce approximately 99% reduction in recoverability of the virus. These results and the results of the studies of virus recovery from the suspension are shown in Table 2. Within the fish fillet samples the average percentage recovery of virus from the eluates of the DEAE Sephadex was high compared to recovery directly from the food filtrates. A correlation coefficient (Bancroft, 1957) of ±0.14 was calculated for the relationship between the change in titer in the irradiated fish filtrates and that of the irradiated suspensions of virus.

Table 3 shows the results of the recovery of polio virus from the DEAE Sephadex column eluates of filtrates of foods stored at 4 and 20°C for 0, 48, 96, 168 hr. Compared to controls virus recovery was generally high from all foods at 4°C with exception of the highly acid foods, cranberry sauce and orange juice. A comparable pattern of recovery was observed in the group of foods held at 20°C except the pork product, which exhibited a higher retention of recoverable virus than the controls. After 48 hr all the foods stored at both temperatures studied exhibited an increasing degree of spoilage.

DISCUSSION

The results of virus recovery from the foods following freeze dehydration suggest that for the foods studied, this processing operation may be expected to reduce polio virus recoverability by about 99.9%. The average percentage of recovery of virus from the food was not appreciably different from the recovery observed in the Hanks' BSS.

The recovery of virus from the freeze dehydrated stock suspension diluted with water was unexpected. Polio virus has been considered as relatively labile during laboratory lyophilization. This suggests a possible difference in the effects on virus survival from the freeze dehydration conditions studied compared to usual procedures employed for virus lyophilization.

The total dosage of Cobalt-60 gamma irradiation given in this study was in the range of those usually considered effective for "pasteurization" of foods (i.e. 300,000 to 600,000 rads). The poor correlation between rate of loss of virus in the fillet samples compared to the virus in the suspensions suggest a significantly greater loss in recoverability of virus from the food. The data show an approximately tenfold greater drop in virus titer in the fish fillet compared to that of the suspen-

Table 2—Recovery of polio virus from suspensions and fish fillets exposed to Cobalt 60 gamma irradiation

Total irradiation in kilorads	Suspension of Virus[1]		Inoculated Fish Fillets[2]	
	Titer of suspension	Titer of DEAE sephadex eluate of suspension	Titer of fish filtrate	Titer of DEAE Sephadex eluate of fish filtrate
0	$4.2 \times 10^{6.3}$	4.2×10^6	4×10^6	5.3×10^5
75	3.2×10^6	2.3×10^6		
150	1.6×10^6	1.6×10^6	6×10^5	1.6×10^5
225	1.4×10^6	9.2×10^5		
300	7.5×10^5	4.2×10^5	2.2×10^5	9.3×10^4
375	3.8×10^5	2.3×10^5	1.1×10^5	3.2×10^4
450	2.2×10^5	2.0×10^5	4.7×10^4	2.0×10^4
525	1.5×10^5	1.7×10^5	3.6×10^4	6.3×10^3
600	1.1×10^6	1.0×10^5	2.3×10	4.0×10^2

[1] Polio virus type 1 strain LS$_c$ 2AB in Eagle's basal medium with Hanks' balanced salt base.

[2] Nine-g samples of Whiting fish fillets inoculated with 1 ml of virus stock suspension containing 5×10^7 plaque forming units (PFU) ml polio virus type 1 strain LS$_c$ 2AB.

[3] Mean PFU/ml of triplicate titration of each sample from three studies.

Table 3.—Recovery of polio virus[1] from DEAE Sephadex column eluates from foods stored at 4 and 20°C

Food	0 hr		48 hr		96 hr		168 hr	
	4°C	20°C	4°C	20°C	4°C	20°C	4°C	20°C
Pork	4.5 × 10⁵ [2]	5.1 × 10⁵	2.3 × 10⁵	1.4 × 10⁵	2.1 × 10⁵	1.5 × 10⁵	1.4 × 10⁵	2.4 × 10⁵
Reconstituted diet Formula	4.8 × 10⁵	4.1 × 10⁵	1.4 × 10⁵	8.0 × 10⁴	2.2 × 10⁵	4.5 × 10⁵	1.3 × 10⁵	3.0 × 10⁴
Cranberry sauce	3.5 × 10⁵	4.0 × 10⁵	2.0 × 10⁵	3.6 × 10⁴	2.7 × 10⁴	2.3 × 10³	2.0 × 10⁴	<1 × 10³
Mayonnaise	4.3 × 10⁵	2.8 × 10⁵	2.1 × 10⁵	3.4 × 10⁵	2.4 × 10⁵	2.1 × 10⁵	1.6 × 10⁵	1.6 × 10⁴
Orange juice	3.0 × 10⁵	4.2 × 10⁵	6.2 × 10⁴	1.6 × 10⁵	5.0 × 10⁴	<1 × 10³	5.7 × 10⁴	<1 × 10³
Virus control[3]	5.0 × 10⁵	4.5 × 10⁵	3.5 × 10⁵	2.1 × 10⁵	5.0 × 10⁵	2.4 × 10⁵	1.0 × 10⁵	2.1 × 10⁵

[1] Nine g samples of food inoculated with 1 ml of virus stock suspension containing 5 × 10⁷ plaque forming units (PFU)/ml polio virus type 1 strain LS, [2] AB in Eagle's basal medium with Hank's salt base.
[2] Mean PFU/ml of triplicate titrations of each sample from two studies.
[3] Stock virus diluted 1:10 in Hanks' balanced salt solution.

sion following 600 kilorad dosage of irradiation. The food environment during irradiation accelerated virus loss compared to loss in the Hanks' BSS suspension. Since all food samples and suspensions were held at approximately 0°C during irradiation, and since recovery of virus from the nonirradiation fish filtrate and suspensions were equivalent, it might be suggested that reactions secondary to irradiation occurring in the fish were detrimental to virus recovery.

The results of the recovery of polio virus from the refrigerated foods suggested that acidity and/or protein content of the food can affect the recoverability of this virus compared to recoverability from suspensions in Hanks' BSS. Otherwise the recovery of virus from refrigerated foods was similar to that reported by Lynt (1966). Similar results were obtained (Heidelbaugh et al., 1967) using Mengovirus in a group of four foods (ranging in pH from 2.4 to 7.1) stored for 14 days at 4°C. In those studies no virus could be detected in the food having pH 2.4 (commercial cola beverage) while the other foods retained in excess of 90% of their original 4.2 × 10⁵ PFU/ml titer after the storage period. In all of these storage studies there was no indication that spoilage of the foods affected virus recovery.

Titers of public health significant virus as high as those studied in this report probably never occur in actual foods. Reductions in virus populations of the order of magnitude reported here may well provide adequate safety margins. Accurate assessment of the impact of food processing operations on the public health aspects of viruses in foods awaits qualitative and quantitative epidemiological data in public health food virology.

It should be emphasized that some of the apparent changes in virus titers following processing operations could result from change in the adherence of virus particles to the food and/or change in the ability of the virus to form plaques under the conditions studied. Measure of virus recovery by methods like those reported here is not necessarily a function of virus infectivity. This suggests the need for a better method for separation of virus particles from foods and the correlation of the results of that method with results of detection of virus infectivity by feeding trials.

In the study of the pork and orange juice, the use of the DEAE Sephadex column was essential for the titration of virus since monolayers of tissue culture cells were frequently destroyed if the food filtrate was not purified by passage through the column. This study also confirms the relatively high percentage of recovery of virus from filtrates passed through a DEAE Sephadex column. This method of separation of infective virus from foods would be useful when highly purified eluates are desired.

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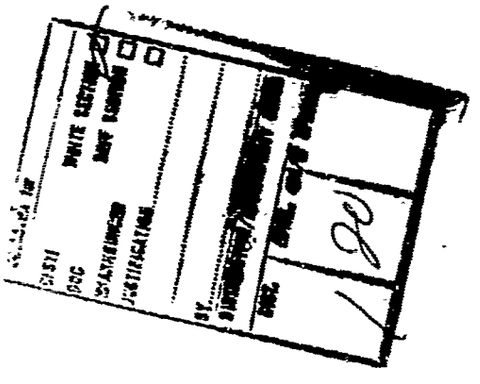

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13. ABSTRACT

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